Claims

1. An *in situ* fluorescence method for on-line monitoring of the state of a sulfur-deprived algal culture to ascertain the culture's ability to produce H_2 under sulfur depletion, comprising:

a) providing a sample of sulfur-deprived algal culture containing photosynthetic components;

b) illuminating said sample with artificial or natural illumination;

c) determining the onset of H_2 photoproduction by measuring the percentage of H_2 in a produced gas phase at multiple times to ascertain the point immediately after the anerobiosis subsequent to the physiological phases of O_2 production and O_2 consumption sequence to obtain data regarding H_2 as a function of time; and

d) determining any abrupt change in the following three *in situ* fluorescence parameters:

i) an abrupt increase in F_t (the steady-state level of chlorophyll fluorescence in light adapted cells);

ii) an abrupt decrease in $F_{m'}$ (the maximal saturating light induced fluorescence level in light adapted cells); and

iii) a precipitous and abrupt decrease in $\Delta F/F_m' = (F_m'-F_t)/F_m'$ (the calculated photochemical activity of photosystem II (PSII)) that signal the full reduction of the plastoquinone pool between PSII and PSI, which indicates the start of anaerobic conditions that in turn induces the synthesis of the hydrogenase enzyme required for subsequent H_2 production, and thereafter slowing down of the abrupt decrease and partial recovery of $\Delta F/F_m'$ signal at least partial oxidation of the plastoquinone pool as the main factor to regulate H_2 production under sulfur depletion.

2. The method of claim 1 wherein said algal culture is any oxygenic photosynthetic microorganism that has a hydrogenase.

3. The method of claim 2 wherein said oxygenic photosynthetic microorganism that has a hydrogenase is green algae.

4. The method of claim 3 wherein said green algae is selected from the group consisting of *Chlamydomonas reinhardtii* ,*Scenedesimus obliguus and Chlorella vulgaris*.

5. The method of claim 4 wherein said green algae is *Chlamydomonas reinhardtii*.

6. The method of claim 5 wherein said abrupt increase in F_t is determined using a fluorometer employing a weak modulated pulse-probe fluorescence method.

7. The method of claim 5 wherein said *in situ* measurement of fluorescence is at or about $\lambda > 710$nm .

8. The method of claim 7 wherein said *in situ* measurement of fluorescence is performed with an optical fiber probe affixed onto a surface of an illuminated glass containing fluorescence excited sample or alternatively with a lens system.

9. The method of claim 7 wherein said *in situ* measurement of fluorescence is performed with a fluorometer set close to the edge of the bioreactor.

10. The method of claim 7 wherein said *in situ* measurement of fluorescence is performed with a lens set close to the edge of the bioreactor.

11. The method of claim 8 wherein a saturated actinic excitation pulse is applied on top of said weak modulated probe pulse.

12. The method of claim 11 wherein said saturated actinic excitation pulse is a 0.8 s pulse $\lambda < 710$nm, 1200 μmol $\cdot$ m^{-2} $\cdot$ s^{-2} PAR) from an 8 V/20W halogen lamp.

13. The method of claim 11 wherein actinic light is about 655nm, 250μmol $\cdot$ m^{-2} $\cdot$ s^{-1} PAR from a LED array for about 2 s for fluorescence induction.

14. The method of claim 12 wherein said saturating actinic excitation pulse is applied on top of a weak modulated probe that flashes at about 3 μs pulses from a 655 nm light-emitting diode at frequencies of from about 600 Hz or 20 kHz.

15. The method of claim 14 wherein efficiency of photochemical conversion of absorbed light energy in PSII is calculated after dark adaptation, where $F_v/F_m = (F_m-F_o)/F_m$.

16. The method of claim 14 wherein efficiency of photochemical conversion of

absorbed light energy in PSII is calculated under steady-state actinic light illumination, where $\Delta F/F_m' = (F_m'-F_t)/F_m'$.